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Research article

Tianeptine induces expression of dual specificity phosphatases and evokes rebound emergence of cortical slow wave electrophysiological activity

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ABSTRACT

Background: The precise mechanism governing the antidepressant effects of tianeptine is unknown. Modulation of brain glutamatergic neurotransmission has been however implicated, suggesting potential shared features with rapid-acting antidepressants targeting **N**-methyl-D-aspartate receptors (NMDAR). Our recent studies suggest that a single subanesthetic dose of NMDAR antagonists ketamine or nitrous oxide (N_2O) gradually evoke 1–4 Hz electrophysiological activity (delta-rhythm) of cerebral cortex that is accompanied by molecular signaling associated with synaptic plasticity (e.g. activation of tropomyosin receptor kinase B (TrkB) and inhibition of glycogen synthase kinase 3β (GSK3β)).

Methods: We have here investigated the time-dependent effects of tianeptine (30 mg/kg, i.p.) on electrocorticogram, focusing on potential biphasic regulation of the delta-rhythm. Selected molecular markers associated with ketamine's antidepressant effects were analyzed in the medial prefrontal cortex after the treatment using quantitative polymerase chain reaction and western blotting.

Results: An acute tianeptine treatment induced changes of electrocorticogram typical for active wakefulness that lasted for 2–2.5 h, which was followed by high amplitude delta-activity rebound. The levels of Arc and Homer1a, but not c-Fos, BdnfIV and Zif268, were increased by tianeptine. Phosphorylation of mitogen-activated protein kinase (MAPK), TrkB and GSK3β remained unaltered at 2-hours and at 3-hours post-treatment. Notably, tianeptine also increased the level of mRNA of several dual specificity phosphatases (*Duspss*) – negative regulators of MAPK.

Conclusion: Tianeptine produces acute changes of electrocorticogram resembling rapid-acting antidepressants ketamine and N_2O. Concomitant regulation of *Dusps* may hamper the effects of tianeptine on MAPK pathway and influence the magnitude of homeostatic emergence of delta-activity and TrkB-GSK3β signaling.

1. Introduction

Tianeptine is an atypical antidepressant with poorly understood mechanism of action [1]. It is structurally related to tricyclic antidepressants but does not inhibit monoamine reuptake [2]. Moreover, unlike many tricyclic antidepressants, tianeptine has very fast elimination both in humans and in rodents ($t_{1/2}$ in mice = 20 min) [3]. Tianeptine preferentially targets μ-opioid receptors with significantly lower affinity towards delta-opioid receptors and negligible binding against other receptors, ion channels and transporters [2]. Indeed, hyperlocomotion and antidepressant-like behavioral responses of tianeptine are abolished in μ-opioid receptor-deficient mice [3]. Animal studies further suggest, that similarly to the well-established rapid-acting antidepressant ketamine [4], modulation of brain glutamatergic neurotransmission may be important for tianeptine's antidepressant effects [5–7].

We have recently shown that subanesthetic doses of ketamine and nitrous oxide (N_2O) – another N-methyl-D-aspartate receptor (NMDAR) antagonist with rapid-acting antidepressant properties [8] – evoke transient increase in electrocorticographic gamma-oscillation (30–100 Hz), followed by delta-activity (1–4 Hz) rebound after their acute

Abbreviations: Arc, activity-regulated cytoskeleton-associated protein; *BdnfIV*, brain derived neurotrophic factor exon IV; *Dusp*, dual specificity phosphatase; GSK3β, glycogen synthase kinase 3β; *Homer1a*, Homer protein homolog 1; MAPK, mitogen-activated protein kinase; SEM, standard error of mean; TrkB, tropomyosin receptor kinase B; Zif268, zinc finger protein 268.

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pharmacological effects subside [9]. Similarly to these alterations of electrocorticogram, regulation of the key molecular-level changes implicated in rapid antidepressant effects show biphasic nature. Namely, both subanesthetic ketamine and N_2O readily and rapidly increase levels of several activity-dependent immediate early genes as well as mitogen-activated protein kinase (MAPK) phosphorylation within the prefrontal cortex [9,10]. Notably, the effects on MAPK phosphorylation reverse once the acute pharmacological effects dissipate and delta-activity emerges [9]. Most importantly, phosphorylation of tropomyosin receptor kinase B (TrkB; receptor for brain-derived neurotrophic factor) and glycogen synthase kinase 3β (GSK3β) become preferentially regulated during the brain state dominated by delta-activity [9].

We have hypothesized that rapid-acting antidepressants share the ability to produce profound, albeit transient, activity in cortical neuronal circuits which triggers homeostatic emergence of delta-oscillations and concomitant regulation of molecular signaling important for antidepressant effects [11]. Considering tianeptine's fast elimination time, its effects on the glutamatergic transmission, and reported suppression of delta activity [3,7] we investigated the acute effects of tianeptine on electrocorticogram, especially its potential time-dependent biphasic effect on delta activity. We further utilized electrocorticographic data to characterize time-points representing ongoing states of cortical excitation and subsequent delta-activity for the assessment of regulation of a panel of immediate early genes *Arc*, *BdnfIV*, *Zif268*, *c-Fos Homer1a, Dusp1, Dusp5 and Dusp 6* and phosphorylation of MAPK, TrkB and GSK3β in response to tianeptine.

2. Materials and methods

2.1. Animals

Eight-to-ten weeks-old male C57BL/6JHsd mice were purchased from Envigo (Netherlands). Animals were single-housed on 12:12 light–dark cycle (lights on at 6:00 a.m.) at 21 ± 2 °C, with *ad libitum* access to food pellets (Scanbur, Sollentuna, Sweden) and water. Mice were habituated to a new environment for at least one week prior to the experiment. Total number of animals used – 28. All experiments were performed according to the Finnish Act on the Use of Animals for Experimental Purposes and the European Communities Council Directive of 24 November 1986 (86/609/EEC), and were approved by the Animal Experiment Committee of the State Provincial Office of Southern Finland. We also adhered to the National Institutes of Health (NIH, USA) guidelines regarding the care and use of animals for experimental procedures.

2.2. Drug treatment

Tianeptine (Tocris Bioscience, UK) was dissolved in saline (0.9% NaCl) and injected intraperitoneally at a single dose of 30 mg/kg. Based on published data [3] this drug dose significantly reduces immobility in the forced swimming test, and increases locomotion. Saline was used as a control. Drug treatments took place 3–6 h after lights on (ZT3-6).

2.3. Electrophysiological recordings

Implantation of the electrocorticographic and the electromyographic electrodes was performed under isoflurane anaesthesia (Attane vet®; induction 5%, maintenance – 1,8–2,5%). Prior to surgery animals received carprofen (Rimadyl®, Pfizer, USA; 5 mg/kg s.c.) as an analgesic and lidocain (Orion Pharma, Finland) for local analgesia at sites of electrodes' implantation. Two brass, gold-plated screws (Dentatus, Sweden) that served as electrodes and anchors were installed into the skull for frontoparietal epidural electrocorticographic recording at the following coordinates: frontal – AP: 2.0 mm, ML: 0.76 mm; parietal: AP: −6 mm, ML: 1.5 mm according to Mouse Brain atlas in Stereotaxic coordinates [12]. The electrodes and custom-made headstages were secured to the skull with dental cement (JC Co., Japan). Silver wire (California Fine Wire, USA) electrodes for electromyogram were placed into the nuchal muscles. Recordings of electrocorticogram and electromyogram were started 9 days post-surgery and continued throughout the course of the experiment in animals' home cages. Baseline recording were conducted 24 h prior to treatment; followed by post-treatment recording for either 120 min (saline; $n = 6$) or 220 min (tianeptine; 30 mg/kg; $n = 7$). Post-treatment recordings were used for spectral and spectrogram analyses.

The electrocorticographic and electromyographic signals were amplified (gain: 5 000 and 500 respectively), filtered (high pass, 0.3 Hz; low pass, 300 Hz), and sampled at 1000 Hz (A-M Systetms, USA). Low-frequency artefacts were further filtered out by digital processing the electrocorticographic signal through a high-pass filter (1 Hz) with Spike2 software (version 8; Cambridge Electronic Devices, Cambridge, UK). The recordings of electrocorticogram were manually verified for the absence of movement artifacts and either processed to spectrogram analysis or assess vigilance state-specific spectral composition (scoring) after drug treatment. Scoring was performed for wakefulness, non-rapid eye movement-, and rapid eye movement sleep that were distinguished as follows: wakefulness – desynchronized electrocorticographic activity accompanied by dampened delta-oscillations; non-rapid eye movement sleep – high amplitude delta-activity in electrocorticogram and low amplitude or absent in electromyogram; rapid eye movement sleep – regular high theta (7–9.5 Hz) activity in electrocorticogram and decreased or absent in electromyogram. The resulting data were used to derive state-specific power spectra. Power spectra were computed using fast Fourier transform with the following parameters: size: 512; epoch length: 4 s; window: Hann; frequency range: 0–100 Hz. Power spectra were normalized using the frequency-wise division of power spectral density values at each vigilance state during treatment phase per power spectral density values of corresponding vigilance state during baseline day. We used non-paired t-tests to compare normalized values between saline and tianeptine groups. To correct for multiple comparisons, we thresholded resulting vectors of t-values to retain only those values that exceeded $p = 0.01$, excluding the remainder from further analysis. Integrated t-values were computed for any contiguous array (cluster) of suprathreshold values. We tested these values for significance against the distribution of integrated t-values of clusters acquired through the permutation-based generation of sets of matrices of t-values, followed by thresholding as described above. We considered clusters with $p < 0.05$ as significant. A detailed description of this procedure can be found in [13].

For spectrogram analysis the data were exported to the Matlab format and filtered with a set of finite-response band-pass filters (step: 1 Hz; window width: 1.4 Hz; filter length: 3000 data points) ranging from 1 to 100 Hz using MatLab 7.5 (MathWorks, Nattick, USA). To make averaged spectrograms filtered data was subjected to a Hilbert transform, normalized frequency-wise to baseline activity and median-averaged over time-and frequency across subjects.

2.4. Dissection of brain samples

Animals were intraperitoneally injected with saline or tianeptine (30 mg/kg) and left undisturbed in their home cages. After indicated time-period, the animals were euthanized using cervical dislocation, followed by decapitation. Next, the brains were rapidly removed and bilateral medial prefrontal cortex dissected on ice, snap-frozen on metal plate cooled with dry ice and stored at −80 °C.

2.5. Quantitative polymerase chain reaction

Extraction of RNA from the tissue was performed using TRIzol (Thermo Scientific, USA) in accordance to manufacturer's instructions and treated with DNAse I (Thermo Scientific, USA). The concentration and purity of extracted RNA was assessed with the Nanodrop 2000

Spectrophotometer (Millipore, USA). Samples with RNA concentration > 100 ng/μl, 260/280 nm ratio > 1.7 and 260/230 nm ratio > 1.8 were processed for cDNA synthesis with the Maxima First Strand cDNA Synthesis Kit and dsDNase mix (K1672, Thermo Scientific, USA). The primers used to amplify specific cDNA regions of the transcripts are shown in Table 1. Primers for *Dusp1*, *Dusp5* and *Dusp6* were designed using PrimerBLAST service (NCBI, USA) and tested for specificity and selectivity. Amplification of cDNA and corresponding control samples was done with Maxima SYBR Green/ROX qPCR Master Mix (2X) (K0221, Thermo Scientific, USA) on the Lightcycler® 480 System (Roche, Switzerland).

Relative quantification of templates was performed by calculating C_q values using the second derivative and dilution series method, with cDNA data being normalized to the joint Gapdh and beta-actin transcripts' levels. The data was compared using two-tailed non-paired Student's T-test for heteroscedastic samples. Leave-one-out analysis was performed to test for robustness of test statistics (data not shown).

2.6. Western blot

For total protein extractions, the brain tissue was homogenized in ice-cold lysis buffer (137 mM NaCl, 20 mM Tris, 1% NP-40, 10% glycerol, 48 mM NaF, H_2O, Pierce™ Protease Inhibitor (Thermo Scientific, USA), Pierce™ Phosphatase Inhibitor (Thermo Scientific, USA)). After a 15-minute incubation, the samples were centrifuged (16,000 g, 15 min, + 4 °C), and the resulting supernatant collected for western blotting. The sample lysates were heated in Laemmli buffer at +100 °C for 3 min, separated in SDS-PAGE under reducing conditions, and blotted on a PVDF membrane. Membranes were incubated overnight with the following primary antibodies: anti-p-TrkB^{Y816} (#4168; 1:1000; Cell signaling technology (CST)), anti-p-GSK3β^{S9} (#9336; 1:1000; CST), anti-p-p44/42-MAPK$^{Thr202/Y204}$ (#9106, 1:1000, CST), and anti-GAPDH (#2118, 1:10000, CST). After the incubation, the membranes were washed with tris-buffered saline with 0.1% Tween 20 and incubated for 1-hour with horseradish peroxidase-conjugated secondary antibody (1:10 000 in non-fat dry milk, Bio-Rad Laboratories). After subsequent washes, the membranes were visualized by enhanced chemiluminescence (ECL Plus, ThermoScientific) and detected with a Biorad ChemiDoc MP camera (Bio-Rad Laboratories).

3. Results

Saline and tianeptine induced acute changes in electrocorticogram that are typical for active wakefulness (Fig. 1(a) and (b)) i.e. suppression of cortical delta- and alpha-activities (10–15 Hz) accompanied by elevation of theta- and gamma-activities). Vigilance state-specific analysis of post-treatment ECoG activity revealed highly significant increase of both total duration and consolidation of wakefulness (Fig. 1(f)) accompanied by concomitant reduction of duration but increased consolidation of NREM sleep (Fig. 1(f)), whereas no changes in REM sleep was observed. Spectral analysis of these vigilance states show an increase in theta-activity during tianeptine-induced wakefulness that lasted at least 2–2.5 h, followed by a transition to sleep-like state with suppressed gamma- and significantly elevated delta-activities (Fig. 1(d) and (e)). Saline increased wakefulness and theta-activity only for approximately 25 min that was likely related to injection stress.

The biphasic ECoG response to acute tianeptine prompted us to select characteristic time-points that likely represent states of cortical excitation and delta-activity rebound for the assessment of regulation of immediate early genes and phosphorylation of MAPK, TrkB and GSK3β in response to tianeptine. To this end, we first collected samples of medial prefrontal cortex 2 h after tianeptine treatment and performed quantitative polymerase chain reaction analyses for *Arc*, *BdnfIV*, *Zif268*, *c-Fos* and *Homer1a* mRNAs. Of these markers, the levels of *Arc* ($p = 1.32e-04$, $n = 6$) and *Homer1a* ($p = 2.7e-04$, $n = 7$) were significantly increased by tianeptine, whereas the mRNA levels for *c-Fos*, *BdnfIV* and *Zif268* remained unaltered (Fig. 2(a)). Phosphorylation of TrkB and GSK3β also remained unchanged in samples collected at this point (Fig. 2(b), Fig. S1(A-D)), i.e. prior to onset of delta-activity. To investigate if tianeptine regulates TrkB and GSK3β phosphorylation at a stage when delta-activity emerges we treated another cohort of mice and collected samples of medial prefrontal cortex at 3-hours post-injections. However, phosphorylation levels of TrkB and GSK3β were again very similar between tianeptine and saline treatment groups (Fig. 2(c), Fig. S1(E-H)). Next, we analyzed phosphorylation of MAPK, as it has been implicated in both homeostatic emergence of delta-activity and antidepressant actions, and several studies suggested tianeptine as potential regulator of MAPK-cascade [17,18]. However, MAPK phosphorylation remained unchanged at both 2-hours and 3-hours after tianeptine administration (Fig. 2(b) and (c)). This prompted us to examine whether tianeptine affects negative regulators of MAPK (*Dusp1*, *Dusp5* and *Dusp6*). Indeed, the mRNA expression of *Dusp1* and *Dusp5* were significantly increased by tianeptine at 2-hours post-injection, while *Dusp6* showed a trend for up-regulation (Fig. 2(d)).

4. Discussion

Here we report that a single dose of tianeptine (30 mg/kg, i.p.)

Table 1
The primers used for quantitative polymerase chain reaction.

Gene	Forward primer (5′→3′)	Reverse primer (5′→3′)
Gapdh[1]	GGTGAAGGTCGGTGTGAACGG	CATGTAGTTGAGGTCAATGAAGGG
Actb[2]	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Arc[3]	AAGTGCCGAGCTGAGATGC	CGACCTGTGCAACCCTTTC
Dusp1[4]	GGGAGAGTGTTTGTTCATTGCC	TCTGCTTCACAAACTCAAAGGC
Dusp5[5]	CAACTTTGGCTTCATGGGACAG	AGGGCTCAGTGTCTGTAAATGG
Dusp6[6]	ACAAAACTGGGCACCTTCATTC	TAGGGAAAGCGACACAGAAGTC
c-Fos[7]	CGGGTTTCAACGCCGACTA	TTGGCACTAGAGACGGACAGA
Zif268[8]	TCCTCTCCATCACATGCCTG	CACTCTGACACATGCTCCAG
Homer1a[9]	GGCAAACACTGTTTATGGACTGG	GTAATTCAGTCAACTTGAGCAACC
BdnfIV[1]	ACCGAAGTATGAAATAACCATAGTAAG	TGTTTACTTTGACAAGTAGTGACTGAA

[1] – [14].
[2] – Primer bank ID: 6671509a1.
[3] – Primer bank ID: 9055166a1.
[4] – Custom made; reference sequence ID: NM_013642.3.
[5] – Custom made; reference sequence ID: NM_001085390.2.
[6] – Custom made; reference sequence ID: NM_026268.3.
[7] – Primer bank ID: 6753894a1.
[8] – [15].
[9] – [16].

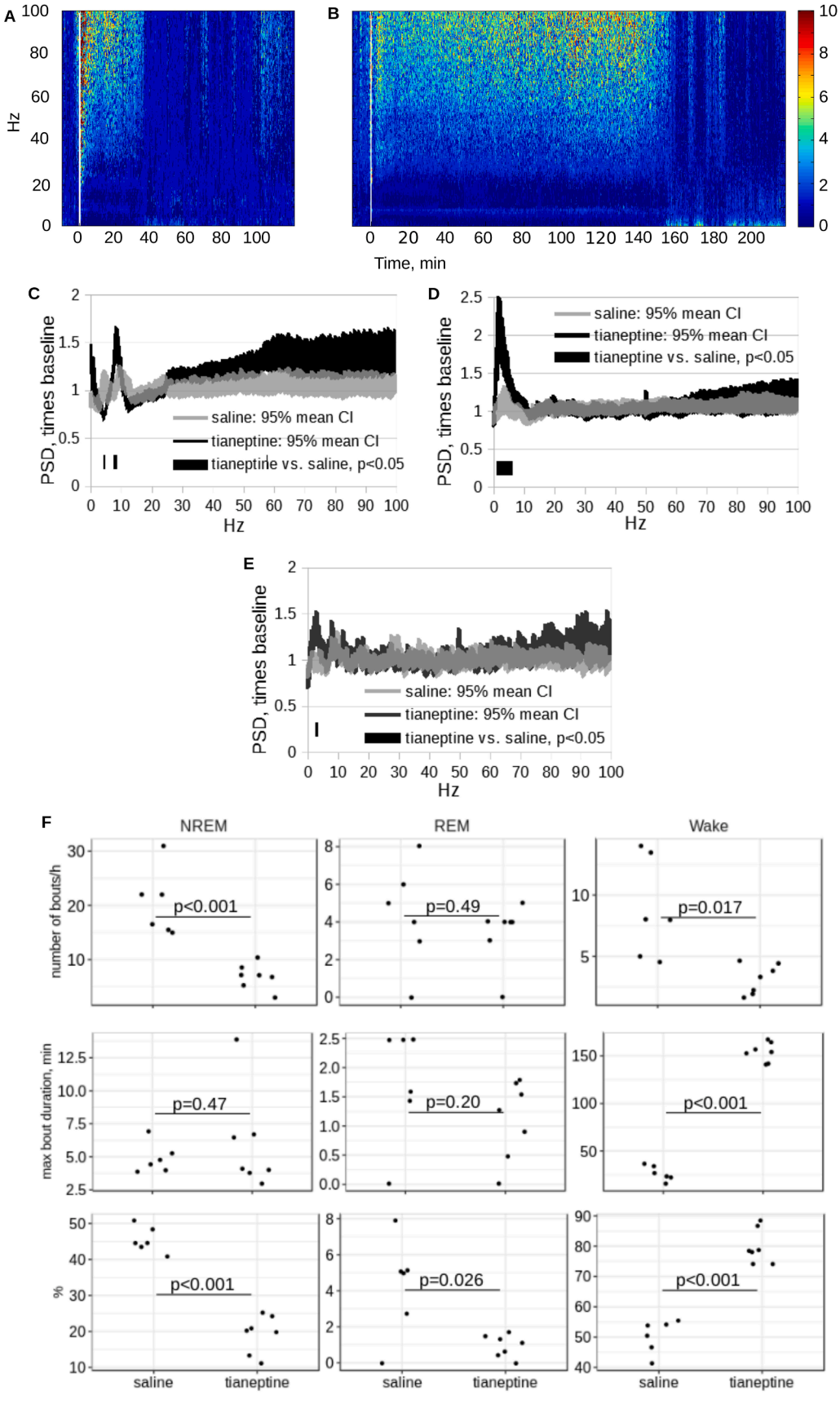

Fig. 1. Electrophysiological effects of tianeptine treatment. Individual spectrograms were normalized and scaled frequency-wise to corresponding baseline, and then they were median-averaged as per treatment: saline (A) and tianeptine (B). Comparison of power spectra acquired during wakefulness (C), non-rapid eye movement- (D), and rapid eye movement sleep (E) stages throughout 120 min after saline- (gray area) or 220 min after tianeptine- (black area) treatment. Data are shown as 95% confidence intervals of the means expressed as a power spectral density (PSD) of treatment phase divided by PSD of baseline. We used the multiple non-paired t-tests for statistical evaluation followed by correction for multiple comparisons using the cluster-based approach; black bars correspond to clusters different from baseline at $p < 0.05$. (F) Tianeptine-induced changes in number or bouts normalized per hour, percentage and the length of the longest bout of wakefulness, NREM and REM sleep states. Non-paired two-tailed t-tests for heteroscedastic samples were used for statistical evaluation; $p < 0.05$ was considered as significant change.

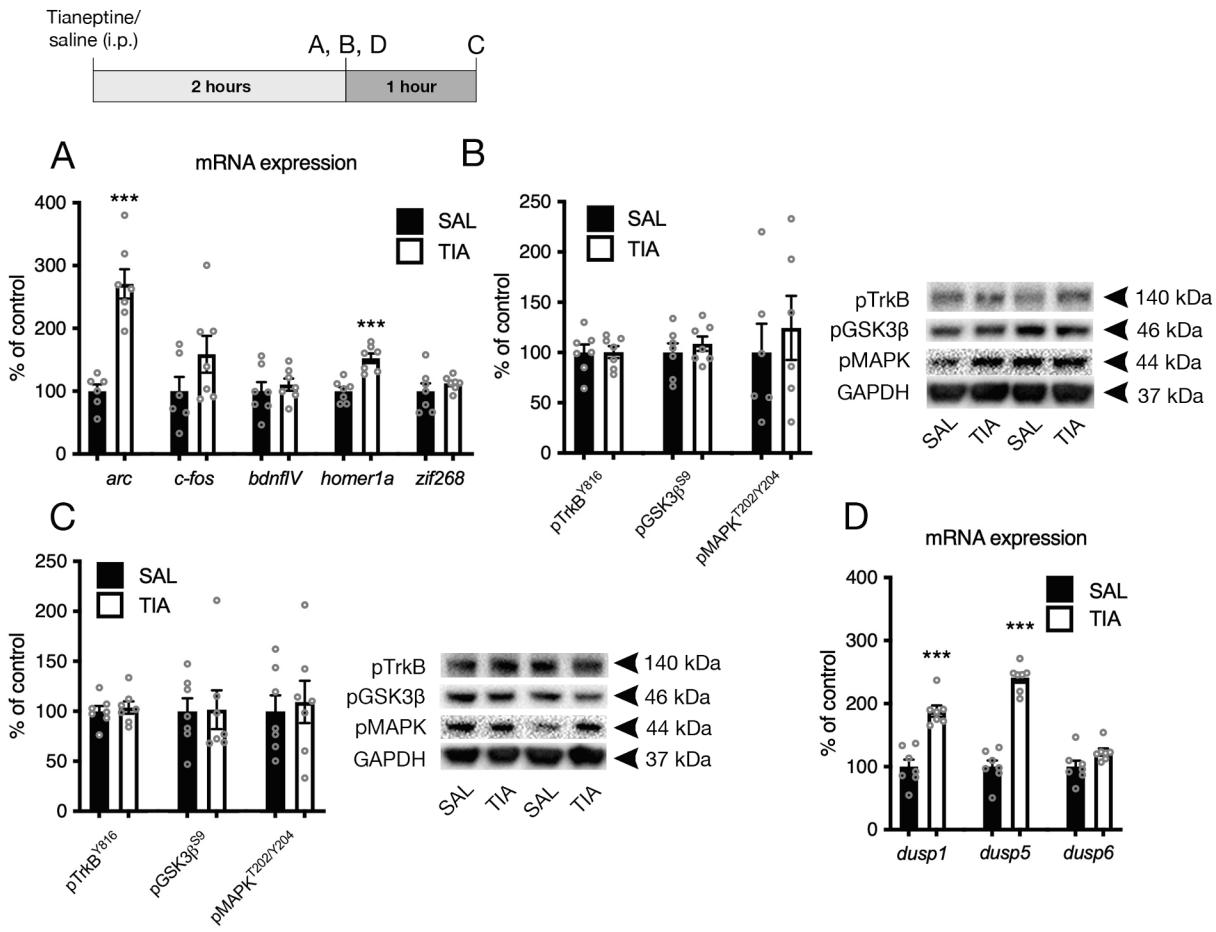

Fig. 2. Effect of tianeptine on expression and phosphorylation of selected molecular markers. Two groups of animals were administered with either saline or tianeptine (30 mg/kg, i.p.), their pre-frontal cortices were isolated 2 h or 3 h later. Gene expression was assessed by qPCR (A and D) and protein phosphorylation by Western blotting (B and C). Data are expressed as % of corresponding saline control (mean ± SEM) and analysed gene-/protein-wise with two-tailed non-paired Student's T-test (n = 6–7; *** – $p < 0.001$). For full western blot images see Fig. S1.

evokes biphasic electrocorticographic responses. Specifically, spectral analysis of electrocorticogram showed an initial suppression of delta- and alpha-activities, and prominent increase of theta- and gamma-oscillations followed by "rebound" high amplitude delta-activity. In terms of suppression of delta-activity our study matches well with previous observations [3,7,19], however, the effect on theta-, alpha- and gamma-activities, to our knowledge have not been reported earlier. These effects of tianeptine remained remarkably long (~2.5 h), provided that its half-life in rodent brain is only about 20 min [3]. Recent study [3] demonstrated, however, that tianeptine's metabolite MC5 has $T_{1/2}$ = 1.5 h in vivo in mice and shows agonist activity towards μ-opioid receptors, albeit 3–5 times lower than tianeptine. Therefore, MC5 could explain the time course of observed changes in spectral composition of electrocorticogram.

Gamma-oscillations have long been associated with activity of local cortical circuits [20]. In turn, expression of μ-opioid receptors in pre-frontal cortex is limited to layers II-III and in particular, to cells with apical dendrites oriented towards superficial layers and morphologically different from pyramidal neurons [21,22]. Although not shown directly in cortex, facilitation of activity of pyramidal cells via inhibition of interneurons by tianeptine was demonstrated on CA1 field of hippocampus [23]. Moreover, similar to tianeptine biphasic electrocorticographic response is typical for NMDA-receptor antagonists ketamine and N_2O [9] and recent studies suggest NMDA-receptor block on gamma-aminobutyric acid-ergic, supposedly somatostatin-positive, cortical interneurons may be the cause of ketamine-evoked elevated gamma-activity [24,25]. Taking into account the inhibitory nature of μ-opioid receptors [21] and their expression pattern in the cortex, interneuron-mediated disinhibition of principal cells could be a plausible mechanism of observed elevation of gamma-activity in response to tianeptine.

After dissipation of these acute effects of tianeptine at approximately 2.5-hours post-injection high-amplitude delta-activity began to dominate the electrocorticographic signal. Such gradual emergence of delta-activity resembles the rebound phase after sleep deprivation [26], as well as response to subanesthetic ketamine and N_2O, albeit the kinetics were somewhat different [9]. Characteristic electrophysiological response to tianeptine and its similarity to that of rapid-acting antidepressants prompted us to examine a panel of immediate early genes implicated in effects of these treatments. First, we assessed expression of a set of molecular targets, whose functions are associated with neuronal excitability and plasticity [27–29], during excitatory phase. We found upregulation of expression of *Arc* and *Homer1* genes, whereas the levels of *c-Fos*, *Bdnf IV* and *Zif268* transcripts remained unaltered. This is in agreement with previous studies of *Arc* [30] and *c-Fos* [31], but not of *Zif268* and *BdnfIV*, whose expression have been reported to elevate after tianeptine treatment [30,32].

ARC and HOMER1 proteins, along with GSK3β and MAPK are involved in post-synaptic trafficking of AMPA receptors [33–36]. We recently demonstrated that subanesthetic dose of ketamine and N_2O rapidly increase phosphorylation of MAPK within the prefrontal cortex during excitation phase, whereas phosphorylation of GSK3β and TrkB preferentially occurred during delta-activity rebound [9]. Therefore, we analyzed phosphorylation of TrkB, GSK3β and MAPK at 2 h and at 3 h after the treatment with tianeptine that corresponded to excitatory and

rebound delta-phases respectively. Unexpectedly, phosphorylation state of these targets remained unchanged in response to tianeptine treatment at both time points. Phosphorylation of MAPK and TrkB in various model systems has fast kinetics (5–40 min post-treatment; [37–40] whereas we collected our samples 120 min after drug administration, suggesting either complete absence of effect on these targets or its dissipation by sampling time. To test this, we analyzed expression of three members of dual specificity phosphatases that are selective negative regulators of MAPK-cascade and revealed elevated levels of Dusp1, Dusp5 and Dusp6 mRNA. Taking into consideration that expression of DUSPs is tightly controlled by MAPK and that several studies suggested tianeptine as potential regulator of MAPK-cascade [17,18], assessment of kinetics of tianeptine-induced phosphorylation of MAPK and expression of Dusps would help to provide mechanistic explanation of observed phenomena. Direct comparison of tianeptine and ketamine may also be informative although reported effects of ketamine on Dusps are contradictory ([41] but see [42]) in rodents. Besides that, as all the interventions mentioned above cause cortical excitation, it is yet to be answered whether observed molecular changes are specific for tianeptine or merely reflect cortical state of vigilance.

Behavioral and electrophysiological effects of tianeptine in general resemble those of morphine and buprenorphine – two well-characterized agonists of μ-opioid receptors. Namely, these substances induce theta- and gamma- electrocorticographic oscillations accompanied by elevated locomotion, followed by sleep-like state [43–46]. Morphine and tianeptine are known to reduce activity of noradrenergic neurons of locus coeruleus [47,48]. Morphine and buprenorphine, unlike tianeptine often induce stupor-like state right after administration accompanied by cortical activation that last>5–10 h. Besides that, withdrawal syndrome typical for morphine , is seldom observed for tianeptine [3]. Such differences can be explained by about a hundred times higher affinity of these compounds to μ-opioid receptors compared to tianeptine [3,49,50], whereas administered dosages are often of the same order [3,44,45]. Limited data are available regarding effects of opioid receptors agonists on DUSPs: an upregulation of *Dusp1* and *Dusp3* expression in hippocampus [51] was shown, whereas the other authors observed downregulation of *Dusp15* in morphine-induced conditioned place preference, and no significant alteration of expression of other Dusps [52]. In addition, physiological effects of morphine are designated by its binding properties to a set of isoforms of μ-opioid receptors [53,54] that, in turn, have distinct localization in the central nervous system [55], for review see [56]. Assessment of isoform-specific properties of tianeptine could help to interpret and possibly resolve these apparently contradictory findings.

Several methodological considerations should be mentioned. First, unchanged level of phosphorylation of MAPK in response to tianeptine could be interpreted as either absence of effect of treatment, or direct consequence of elevated levels of phosphatases or as suboptimal selection of time points of measurement of pMAPK quantity. Distinction between these reasons would require quantification of DUSP1 and DUSP5 proteins and/or analysis of their other known targets as well as selection of additional time points at earlier stages of treatment to quantify pMAPK amount and assessment of ratio between pMAPK and its non-phosphorylated form. In addition, interpretation of the electrophysiological data would benefit from co-localization study of *Arc*, *Homer1a* and *Dusps* with characteristic markers of subpopulations of cortical neurons. Finally, assessment of both the ECoG and molecular markers in the same animals, and the use of more comprehensive dosing protocols, may be valuable for future studies.

In conclusion, an atypical antidepressant tianeptine produces acute changes in electrocorticogram in adult rodents resembling sleep deprivation, subanesthetic ketamine and N_2O. Concomitant regulation of transcription of *Dusp* genes may hamper the effects of tianeptine on MAPK pathway and thereby influence the magnitude of homeostatic emergence of delta-activity and TrkB-GSK3β signaling. Studies directly comparing the EEG responses acutely elicited by tianeptine and the rapid-acting antidepressants could better highlight mechanistic similarities, and differences, between these clinically used treatments against depression.

CRediT authorship contribution statement

Stanislav V. Rozov: Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Supervision. **Marko Rosenholm:** Formal analysis, Investigation, Writing - review & editing, Supervision. **Simo Hintikka:** Investigation. **Tomi Rantamäki:** Conceptualization, Resources, Writing - original draft, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflicts of interests

T.R. is listed as a co-inventor on a patent application wherein new EEG monitoring tools potentially enabling the development of rapid-acting antidepressants and the efficacy monitors thereof are disclosed. T.R. has assigned his patent rights to the University of Helsinki but will share a percentage of any royalties that may be received by the University of Helsinki.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neulet.2021.136200.

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